

WHAT IS CLAIMED IS:

1. A replicase complex comprising an HCV NS5B protein, a linear nucleic acid template and a complementary nucleic acid primer which is annealed to the 3' terminus of the template, wherein the template is at least three nucleotides and the primer is two or three
5 nucleotides, and the template and primer do not form a stable duplex in solution in the absence of the HCV NS5B protein.

2. The replicase complex of claim 1, wherein the primer sequence is selected from GG, GC, CG, CC, GU, UG, CA, and AC.

3. The replicase complex of claim 1, wherein the base of the first nucleotide of the primer is a guanine.

4. The replicase complex of claim 1, which is in contact with an inhibitory
15 compound of the HCV NS5B protein.

5. An assay system for detecting HCV replicase activity comprising
an enzymatically active amount of HCV NS5B protein;
an RNA template which comprises at least five nucleotides; and
20 an RNA primer which is complementary to the 3' terminus of the template and comprises two or three nucleotides, wherein the template and primer do not form stable duplex in solution in the absence of the NS5B protein, ATP, GTP, CTP, and UTP nucleoside triphosphates (NTPs), wherein only one of the NTPs or the primer is radiolabeled, and an assay buffer that permits replication activity of NS5B.

6. The assay system of claim 5, wherein the base of the first nucleotide of the primer is a guanine.

7. The assay system of claim 5, wherein the NS5B is in contact with an
30 inhibitory compound of the NS5B protein.

8. The assay system of claim 5, wherein the NS5B is soluble and has enzymatic activity when expressed in *Escherichia coli*.

9. The assay system of claim 5, wherein the RNA template lacks any stable secondary structure at the 3' terminus.

5 10. The assay system of claim 5, wherein the radiolabel is a phosphate isotope.

11. The assay system of claim 5, wherein the radiolabeled NTP is α -³³P-NTP which hydrogen bonds to a nucleotide of the template.

10 12. The assay system of claim 5, wherein the assay buffer comprises 50 mM HEPES (pH 7.3), 10 mM β -mercaptoethanol, 50 mM NaCl, and 5 mM MgCl₂, the template RNA is 5 μ M, the primer is 10 μ M, the HCV NS5B protein is 3 to 5 μ M, and the NTP substrate is 100 μ M.

15 13. A method for detecting HCV replicase activity, comprising detecting a nucleic acid synthesized by an HCV NS5B protein on a linear RNA template which comprises at least three nucleotides and an RNA primer which comprises two or three nucleotides, wherein the primer basepairs to the 3' end of the template and the RNA template and the primer do not form a stable duplex in the absence of HCV NS5B protein, in the presence of
20 ATP, GTP, CTP, or UTP (NTPs), wherein only one of the NTPs is radiolabeled, and an assay buffer that supports replication activity of NS5B.

25 14. The method according to claim 13, wherein detecting the nucleic acid synthesized by NS5B comprises evaluating an autoradiograph of reaction products separated by gel electrophoresis.

15. The method according to claim 13, wherein the NS5B is a soluble NS5B expressed in *Escherichia coli*.

30 16. The method according to claim 13, wherein the base of the first nucleotide of the primer is a guanine.

17. The method according to claim 13, wherein the radiolabel is a phosphate isotope.

18. The method according to claim 13, wherein the radiolabeled NTP is an α - ^{33}P -
5 NTP that hydrogen bonds to the template.

19. The method according to claim 13, wherein the assay buffer comprises 50 mM HEPES (pH 7.3), 10 mM β -mercaptoethanol, 50 mM NaCl, and 5 mM MgCl_2 , the template RNA is 5 μM , the primer is 10 μM , the HCV NS5B protein is 3-5 μM and the NTP is 100
10 μM and the assay is performed at 30°C.

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